INFECTION AND IMMUNITY, Jan. 1989, p. 213–218
0019-9567/89/010213-06$02.00/0

Vol. 57, No. 1

A Protease of *Bacteroides gingivalis* Degrades Cell Surface and Matrix Glycoproteins of Cultured Gingival Fibroblasts and Induces Secretion of Collagenase and Plasminogen Activator

VELI-JUKKA UITTO,[1]* HANNU LARJAVA,[2] JYRKI HEINO,[2] AND TIMO SORSA[3]

Department of Oral Biology, University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z7,[1] and Department of Medical Chemistry, University of Turku, Turku,[2] and Department of Medical Chemistry, University of Helsinki, Helsinki,[3] Finland

Received 27 June 1988/Accepted 21 September 1988

To assess the direct effects of *Bacteroides gingivalis* on periodontal cells, human gingival fibroblasts were cultured in the presence of *B. gingivalis* extracts or a trypsinlike enzyme partially purified from the bacteria by chromatography on benzamidine-Sepharose and Sephacryl S-200. Analysis of cell surface glycoproteins by the periodate-[^{3}H]borohydride labeling technique combined with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)–fluorography demonstrated that fibronectin and some other high-molecular-weight cell surface glycoproteins were degraded by a 35,000-M_r(35K) *B. gingivalis* protease. Immunostaining of the fibroblast cultures showed degradation of intercellular matrix fibronectin by the 35K protease. The pattern of fibronectin degradation was monitored by examining the reaction products with the SDS-PAGE–immunoblotting technique. The protease degraded fibronectin rapidly and more extensively than did corresponding amounts of pancreatic trypsin. Collagenase secretion by the fibroblasts was assayed by incubating cell culture medium with soluble type I [^{3}H]collagen at 25°C followed by SDS-PAGE–fluorography analysis of the reaction products. The medium was also assayed for plasminogen activator activity by using a casein-agarose diffusion plate assay. The fibroblasts cultured with the 35K protease secreted increased amounts of collagenase and plasminogen activator into the medium. The results suggest that periodontal infection by *B. gingivalis* causes proteolytic damage of the host cell surface structures. Concomitantly, *B. gingivalis* may induce the cells to degrade their pericellular matrix.

Bacteroides gingivalis has been implicated as an important pathogen in the establishment and progression of periodontal diseases (15, 27). In destructive forms of the disease, the organism is frequently found in the subgingival microflora (26). Furthermore, inoculation of nonhuman primates with *B. gingivalis* has been shown to enhance periodontal breakdown (5). The virulence of this organism is believed to arise from several factors. For instance, specific surface structures protect the bacteria from the host defense (30) and provide the means for adherence and colonization (17). In addition, *B. gingivalis* possesses an unusual arsenal of hydrolytic enzymes by which the organism can utilize many kinds of tissue macromolecules for its metabolic needs (15, 27). It is not understood which part the hydrolytic enzymes and other substances, such as lipopolysaccharides and metabolic byproducts, play in the virulence of *B. gingivalis*. In theory, these factors may exert several kinds of direct and indirect deleterious effects on both cellular and matrix elements of human tissue (15, 27). We have recently reported partial purification of a major proteolytic enzyme from *B. gingivalis* (28). The enzyme has an M_r of 35,000 (35K protease) and shows specificity for arginine-containing substrates. In this study, we investigated the direct effects of this *B. gingivalis* protease on human fibroblasts.

MATERIALS AND METHODS

Preparation of *B. gingivalis* samples. *B. gingivalis* ATCC 33277 was cultured anaerobically for 3 days on bacteriological agar (Oxoid Ltd., Hampshire, England) containing 5% horse blood, 0.5% yeast extract, 0.5 mg of menadione per ml, 500 mg of cysteine per liter, and 0.2% glucose. The bacterial mass was harvested in phosphate-buffered saline (PBS) and washed twice by low-speed centrifugation. The cells were homogenized with a Branson B3 sonicator until more than 90% of the bacteria were disrupted as determined by examination under a light microscope. The homogenate was centrifuged at 11,000 × *g*. The supernatant was passed through a benzamidine-Sepharose affinity chromatography column, and the bound fraction was eluted at pH 2.0. Following adjustment of the pH of the eluent to 7.5, it was passed through a Sephacryl S-200 gel filtration chromatography column (28). The fraction eluted in the 35,000-M_r region showed the highest activity against benzoyl-arginyl-paranitroanilide, a trypsin substrate, and was used in the experiments. The purification was 12-fold, and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) silver stain analysis showed some minor contaminating bands in the high-molecular-weight region. In this text, the enzyme is designated as the 35K protease. The crude *B. gingivalis* extracts (11,000 × *g* supernatant fraction of the sonicate) with or without heat treatment (10 min at 100°C) were included in the experiments on cell morphology and fibronectin distribution.

Fibroblast cultures. Fibroblasts were cultured from a gingival biopsy obtained from a person with a clinically healthy periodontium. Cells from passage 3 were cultured in Dulbecco modified Eagle medium supplemented with 10% fetal bovine serum, 20 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer, 23 mM glucose, 50 μg of streptomycin sulfate per ml, and 100 IU of penicillin per ml.

Analysis of cell surface glycoproteins. The medium of confluent cultures of fibroblasts was removed, and the cell

* Corresponding author.

layer was washed three times with PBS without fetal bovine serum. Pancreatic bovine *N*-tosyl-L-phenylalanine chloromethyl ketone-treated trypsin (Sigma type XIII) or the purified *B. gingivalis* 35K protease in Dulbecco modified Eagle medium was added to the cultures to a concentration of 16 U/ml. The proteolytic activity was determined by using benzoyl-arginyl-paranitroanilide (1 mM) as the substrate. One activity unit is defined as the amount of enzyme producing an optical density change at 405 nm of 0.001/min at 37°C. Following incubation for 1 h at 37°C, the cells were harvested with a rubber policeman and washed twice with PBS with low-speed centrifugation. The cell surface glycoproteins were labeled by the periodate-[^{3}H]sodium borohydride technique as described by Andersson and Gahmberg (1). The proteins were analyzed on SDS-PAGE by the Laemmli method (9), followed by the fluorography technique as described by Laskey and Mills (12).

Assay of fibronectin degradation. Human plasma fibronectin purified by affinity chromatography was obtained from A. Vaheri, Department of Virology, University of Helsinki, Helsinki, Finland. Pancreatic trypsin or the 35K protease was incubated at 37°C with 4 μg of fibronectin in 50 mM Tris hydrochloride (pH 7.3) with various enzyme amounts and incubation times. The degradation products of fibronectin were analyzed by SDS-PAGE with 6% gels, followed by immunoblotting (8). The peptides were transferred from the gels onto a nitrocellulose sheet (Millipore HAWP 000 001) (33). The sheet was then saturated with 0.05% Tween 20 in PBS. Anti-fibronectin immunoglobulin G (IgG) (Dakopatts, Copenhagen, Denmark) at a 1:100 dilution was added for 1 h. Following extensive washing of the sheet with PBS, alkaline phosphatase-conjugated anti-rabbit IgG (Oriola Diagnostics, Helsinki, Finland) was added at a dilution of 1:100 for 1 h. Following further extensive washing with PBS, the sheet was incubated at 4°C for 3 min with α-naphthyl phosphate and 4-aminodiphenylamine diazonium sulfate.

Immunostaining of fibronectin in fibroblast cultures. Confluent fibroblast cultures were incubated for 16 h in Dulbecco modified Eagle medium without serum in the presence of 6 U of the 35K protease per ml or crude extracts of *B. gingivalis* at a final concentration of 100 μg of protein per ml (14). The cultures were fixed with 3.5% paraformaldehyde in PBS, made permeable with Triton X-100, and immunostained with anti-fibronectin IgG at a 1:50 dilution as the primary antibody and fluorescein isothiocyanate-conjugated anti-rabbit IgG (Oriola Diagnostics) at a 1:100 dilution as the secondary antibody (36).

Collagenase assay. Fifty microliters of culture medium was incubated with soluble [^{3}H]proline-labeled chicken type I collagen in 0.05 M Tris hydrochloride buffer (pH 7.8) containing 0.2 M NaCl and 0.005 M $CaCl_2$ for 18 h at 25°C as described in a previous paper (34). The specific radioactivity of the collagen was 2×10^4 dpm/mg. To activate latent collagenase in the culture medium, 1 mM *p*-aminophenylmercuric acetate was added. The activation effect of plasminogen activator in the culture medium through the formation of plasmin was studied by adding 50 μg of plasminogen per ml to the collagenase incubation mixture. The degradation products of collagen were analyzed by SDS-PAGE (9) followed by fluorography (12).

Plasminogen activator assay. Twenty microliters of culture medium was incubated at 28°C in wells made in agarose plates containing plasminogen and casein as described earlier (23). When plasminogen is converted to plasmin by the plasminogen activator, it degrades casein and forms a clear lysis disk in the gel which is proportional to both the plasminogen activator activity of the sample and the time of diffusion. The casein-agarose plates without plasminogen were included to provide a control of nonspecific protease activity in the samples. Human urokinase was used as the standard.

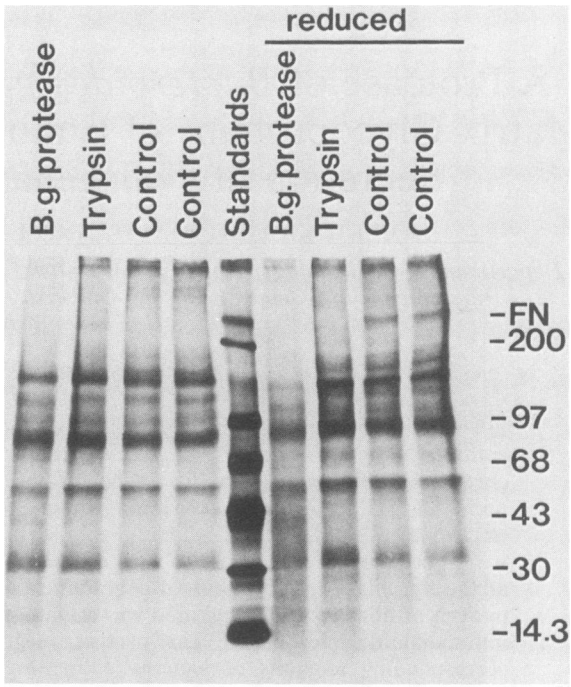

FIG. 1. SDS-PAGE of radioactively labeled gingival fibroblast surface glycoproteins. Pancreatic trypsin or the *B. gingivalis* 35K protease (16 U/ml) was added to confluent cell cultures for 60 min. The glycoproteins were labeled by the periodate-[^{3}H]sodium borohydride technique and then separated on a 5 to 22% polyacrylamide gel. The controls in lanes 3 and 8 were fibroblasts incubated with heat-treated *B. gingivalis* protease, and those in lanes 4 and 9 were cells without enzymes added. Fn, Fibronectin.

Materials. Tritiated sodium borohydride (7 Ci/mmol) was obtained from Amersham Corp., Arlington Heights, Ill. The other reagents and chemicals, if not otherwise stated, were obtained from Sigma Chemical Co., St. Louis, Mo.

RESULTS

Cell surface glycoproteins. Following culture with equal activity units of the *B. gingivalis* 35K protease or pancreatic trypsin, the fibroblasts were surface labeled by a periodate-[^{3}H]borohydride technique. The proteins were then separated by SDS-PAGE with or without reduction. Figure 1 shows a fluorograph of the gel. The principal effect of pancreatic trypsin was degradation of one surface glycoprotein. It was identified as fibronectin on the basis of molecular weight (about 220,000 when reduced) and Western blot (immunoblot) analysis. The 35K protease produced more extensive degradation of the cell surface structures. Several glycoproteins with M_rs of >100,000 were degraded. Fibronectin was completely degraded, and one of the major glycoproteins, with an M_r of about 140,000, was partially destroyed. Upon reduction, diffused bands corresponding to peptides with M_rs of 20,000 to 60,000 were detected in the gel.

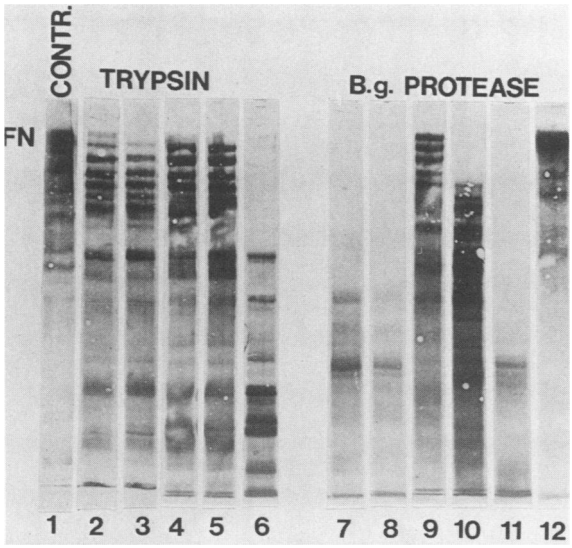

FIG. 2. Degradation of plasma fibronectin (Fn) by pancreatic trypsin and the *B. gingivalis* 35K protease. Following incubation at 37°C, the samples were run on SDS-PAGE using 6% gels. The fibronectin fragments were transferred to nitrocellulose membrane and visualized by staining with anti-fibronectin IgG–alkaline phosphatase-conjugated anti-rabbit IgG. The incubation times and enzyme concentrations were as follows: lane 1, control, 19 h; lane 2, trypsin (30 U/ml), 5 min; lane 3, trypsin (30 U/ml), 60 min; lane 4, trypsin (0.6 U/ml), 19 h; lane 5, trypsin (3.0 U/ml), 19 h; lane 6, trypsin (30 U/ml), 19 h; lane 7, 35K protease (30 U/ml), 5 min; lane 8, 35K protease (30 U/ml), 60 min; lane 9, 35K protease (0.6 U/ml), 9 h; lane 10, 35K protease (3.0 U/ml), 19 h; lane 11, 35K protease (30 U/ml), 19 h; lane 12, heat-inactivated 35K protease (30 U/ml), 19 h.

Degradation of exogenous and matrix fibronectin. Purified plasma fibronectin was incubated with equal activity units of the 35K protease or pancreatic trypsin for various time periods. At a low concentration of both proteases, fibronectin was degraded to several peptides, most of them showing M_rs between 60,000 and 200,000 (Fig. 2, lanes 4 and 9). At higher concentrations, the 35K protease showed more extensive degradation of fibronectin than did trypsin (Fig. 2, lanes 5, 6, 10, and 11). Furthermore, its degradation was very rapid. Within 5 min, trypsin produced fibronectin fragments, most of which had relatively high M_rs. During the same time period, the 35K protease almost completely degraded the fibronectin molecules (Fig. 2, lanes 2 and 7). Heating the protease to 100°C completely destroyed its activity. The fibronectin in the fibroblast matrix was detected by immunostaining following culture with the 35K protease. Almost complete degradation of the intercellular fibronectin network was observed (Fig. 3, panel E). For comparison, the fibroblasts were also incubated with crude *B. gingivalis* extracts. Extensive degradation of the extracellular fibronectin was evident (Fig. 3, panel C). In contrast, cells treated with either the crude extracts or the purified 35K protease showed positive staining for fibronectin. Heating of the crude extracts or the protease totally prevented degradation of the extracellular fibronectin (Fig. 3, panels B and D).

Cell morphology. Under phase-contrast microscopy, the fibroblasts cultured with the 35K protease for 16 h showed no marked alterations. They were well attached and had an orientation similar to that of the control cells, although some widening of the intercellular space was recorded (Fig. 3, panels J, F, and I). In contrast, the cultures with crude

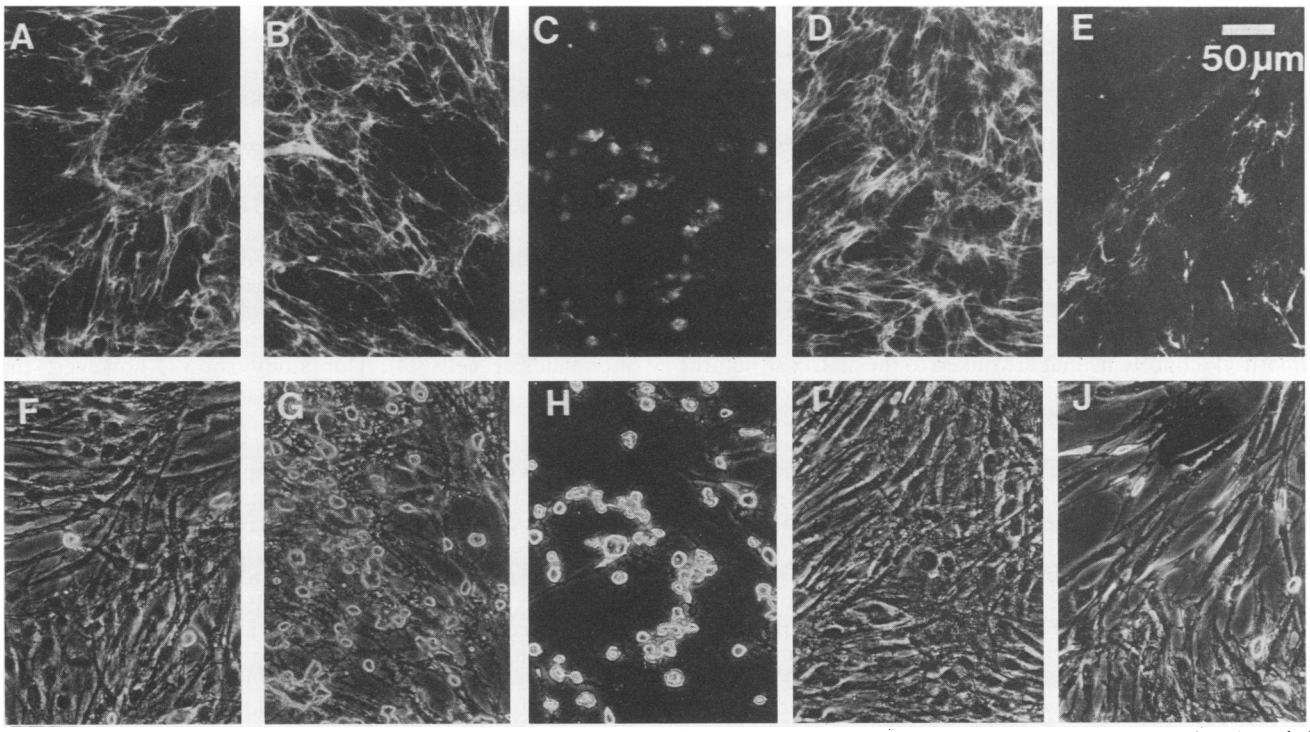

FIG. 3. Immunofluorescence localization of fibronectin (panels A to E) and the corresponding phase-contrast microscope view (panels F to J) of fibroblasts cultured in the presence of *B. gingivalis* preparations. Confluent cultures of human gingival fibroblasts were exposed for 16 h to the preparations. Panels: A and F, control cultures; B and G, heat-treated crude extracts of *B. gingivalis*; C and H, unheated *B. gingivalis* extracts; D and I, heat-treated *B. gingivalis* 35K protease; E and J, unheated *B. gingivalis* 35K protease.

TABLE 1. Effect of *B. gingivalis* 35K protease on plasminogen activator activity of cultured fibroblasts[a]

Concn of 35K protease (U/ml)	Mean ± SD plasminogen activator activity (Ploug U/ml)
0 (controls)	0
0.6	0
1.2	0.6 ± 0.1[b]
3.0	2.8 ± 0.4[b]
6.0	7.8 ± 1.2[b]

[a] Samples of 2×10^5 cells each were cultured to confluency in Dulbecco modified Eagle medium containing 10% fetal bovine serum. The medium was changed to 0.5% serum, and the 35K protease was added. Twenty microliters of each medium sample was assayed for plasminogen activator activity on day 2 by using the casein-agarose gel diffusion assay in the presence of SDS. No caseinolysis was detected with the protease alone or if plasminogen was not included in the gel. The values represent samples from four parallel cultures.
[b] Differs significantly from the control ($P < 0.01$; Student t test).

extracts showed rounding, detachment, and loss of fibroblasts (Fig. 3, panel H). Heat treatment of these extracts only partially prevented this effect. These latter cultures showed more cells, many of which appeared to be attached and oriented normally. However, numerous rounded and detached fibroblasts were also present (Fig. 3, panel G).

Secretion of plasminogen activator and collagenase. In the presence of low levels of the 35K protease, the fibroblasts secreted no detectable levels of plasminogen activator during 2 days of culture in a medium containing 0.5% serum. However, the 35K protease stimulated the fibroblasts to secrete plasminogen activator. The effect was dose dependent (Table 1). During 2 days of culture in the presence of the 35K protease, the fibroblasts also secreted collagenase (Fig. 4, lanes 1 to 6). Secretion of collagenase was clearly diminished following removal of the protease from the culture medium. Collagenase in all cultures was in an entirely latent form and appeared only when activated with aminophenylmercuric acetate, a sulfhydryl reagent. Collagenase in the medium was also activated when plasminogen was added to the collagenase incubation. The activity, however, was only 40 to 50% of that of the aminophenylmercuric acetate-activated samples (data not shown).

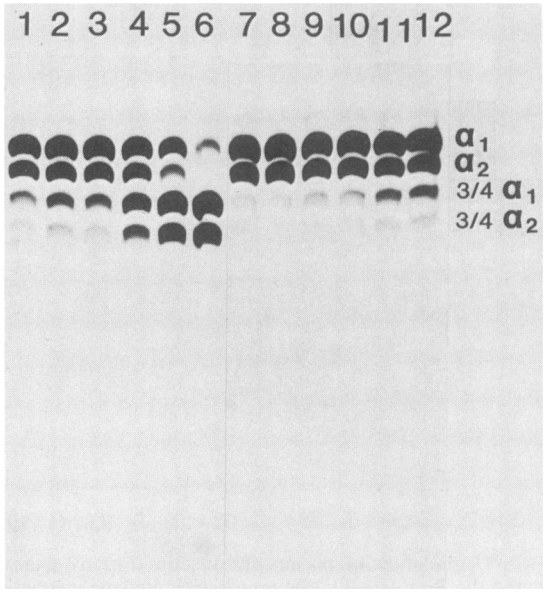

FIG. 4. Collagenase secretion of human gingival fibroblasts treated in culture with the *B. gingivalis* 35K protease. Confluent fibroblasts were cultured in the presence of 0.5% serum and the protease for 2 days (lanes 1 to 6). Thereafter, the medium was changed and the cultures were continued for 3 days without the protease (lanes 7 to 12). Unconcentrated medium was incubated for 18 h at 25°C with soluble [^{3}H]proline-labeled type I collagen in the presence of 1 mM aminophenylmercuric fluoride. The reaction mixture was run on SDS-PAGE and processed for fluorography. The protease concentrations were as follows: lanes 1, 2, 7, and 8, 0 U/ml (controls); lanes 3 and 9, 1 U/ml; lanes 4 and 10, 2 U/ml; lanes 5 and 11, 5 U/ml; lanes 6 and 12, 10 U/ml. The positions of the collagen alpha chains and their 3/4-length degradation fragments are indicated.

DISCUSSION

The interaction of a cell with the extracellular matrix or another cell is largely mediated by cell surface molecules (7, 40). During recent years, a number of cell surface receptors termed integrins has been discovered. These receptors are dimeric glycoproteins that are linked to the matrix structures through their cell surface domains and to the cytoskeletal elements through their intracellular domains (7, 21). Fibronectin is always present on the surface of normal mature fibroblasts and in the extracellular matrix of connective tissue (29). It is a major glycoprotein responsible for cellular behavior, e.g., attachment, spreading, proliferation, and synthetic activity (6, 31, 37, 40). In this study, we found that a trypsinlike protease, abundant in *B. gingivalis*, is capable of effectively destroying both fibronectin and some other fibroblast surface glycoproteins. Some vertebrate proteases, such as trypsin, chymotrypsin, elastase, and plasmin, have been shown to degrade cell surface fibronectin (2, 31, 32, 37). Concomitantly, DNA synthesis and cell proliferation are increased by the proteases (3, 20, 32). It is not known what specific cellular changes result from the action of the *B. gingivalis* proteases or how reversible the damage is. It has been shown that treatment of gingival fibroblasts with bacterial plaque extracts leads to degradation of some cell surface proteins but that the fibroblasts are able to partially repair the damage (10).

One important aspect of cellular behavior in periodontal diseases is the production of proteolytic enzymes, such as collagenase (19). Collagenase in inflamed human periodontal tissues has been found to originate predominantly from human cells and not from the infecting microorganisms (35). It has been suggested that the major route for bacterial induction of the production of collagenase and prostaglandin E_2 in fibroblasts is through the release of interleukin-1 from mononuclear cells (4). Our study shows, however, that protease from *B. gingivalis* is capable of directly inducing fibroblasts to secrete collagenase. An earlier study found that trypsin and plasmin induce collagenase from cultured fibroblasts (38). Production of plasminogen activator is usually greatly enhanced in fibroblastic cells transformed by viruses or chemicals (18, 22, 24, 25). Excessive production of plasmin may trigger reactions that lead to pathological changes in cells. It has been suggested that plasmin degrades fibronectin and laminin in the cell attachment sites, thus releasing cells from contact inhibition (13, 16, 31). In addition, plasmin may activate latent forms of collagenases specific for interstitial collagens (39) and type IV (basement membrane) collagen (25). This could further enhance matrix degradation in the pericellular area.

The role of the 35K protease in this study and of other hydrolytic enzymes in the virulence of *B. gingivalis* is a subject for further research. It appears, however, that the

35K protease has the potential to damage cell surface structures and matrix proteins. Moreover, the protease may directly induce production of collagenase and plasminogen activator in fibroblasts. Obviously, in addition to proteases there are other significant factors associated with *B. gingivalis* that can exert direct deleterious effects on host cells. The morphology of fibroblasts cultured in the presence of heated *B. gingivalis* extracts was markedly altered despite the presence of an apparently normal fibronectin network. We have previously found that lipopolysaccharides are probably the main factor in the inhibition of fibroblast growth by *B. gingivalis* (11). Furthermore, we found that lipopolysaccharides of *B. gingivalis* also induce plasminogen activator secretion in fibroblasts and epithelial cells (Uitto and Larjava, unpublished data).

ACKNOWLEDGMENTS

This study was supported by a development grant from the Medical Research Council of Canada and a grant from the Academy of Finland.

LITERATURE CITED

1. **Andersson, L. C., and C. G. Gahmberg.** 1978. Surface glycoprotein of human white blood cells. Analysis of surface labeling. Blood **52**:57–67.
2. **Atkinson, P. H., and J. Hakimi.** 1981. Alterations in glycoproteins of the cell surface, p. 191–239. *In* W. J. Lennarz (ed.), The biochemistry of glycoproteins and proteoglycans. Plenum Publishing Corp., New York.
3. **Blumberg, P. M., and P. W. Robbins.** 1975. Effect of proteases on activation of resting chick embryo fibroblasts and on cell surface proteins. Cell **6**:137–147.
4. **Heath, J. K., S. J. Atkinson, R. M. Hembry, J. J. Reynolds, and M. C. Meikle.** 1987. Bacterial antigens induce collagenase and prostaglandin E_2 synthesis in human gingival fibroblasts through a primary effect on circulating mononuclear cells. Infect. Immun. **55**:2148–2154.
5. **Holt, S. C., J. Ebersole, J. Felton, M. Brunsvold, and K. S. Kornman.** 1988. Implantation of *Bacteroides gingivalis* in nonhuman primates initiates progression of periodontitis. Science **239**:55–57.
6. **Hsieh, P., and L. B. Chen.** 1983. Behaviour of cells seeded in isolated fibronectin matrices. J. Cell Biol. **96**:1208–1217.
7. **Hynes, R. O.** 1987. Integrins: a family of cell surface receptors. Cell **48**:549–554.
8. **Jalkanen, M., and S. Jalkanen.** 1983. Immunological detection of proteins after isoelectric focusing in thin layer agarose gel: a specific application for the characterization of immunoglobulin diversity. J. Clin. Lab. Immunol. **10**:225–79–84.
9. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
10. **Larjava, H., and M. Jalkanen.** 1984. Cell surface glycoconjugates of gingival fibroblasts exposed to dental plaque extract. J. Periodont. Res. **19**:469–482.
11. **Larjava, H., V.-J. Uitto, E. Eerola, and M. Haapasalo.** 1987. Inhibition of gingival fibroblast growth by *Bacteroides gingivalis*. Infect. Immun. **55**:201–205.
12. **Laskey, R. A., and A. D. Mills.** 1974. Quantitative film detection of ^3H and ^{14}C in polyacrylamide gels by fluorography. Eur. J. Biochem. **56**:335–340.
13. **Liotta, L. A., R. H. Goldfarb, and V. P. Terranova.** 1981. Cleavage of laminin by thrombin and plasmin: alpha thrombin cleaves the beta chains of laminin. Thromb. Res. **21**:663–673.
14. **Lowry, O. H., N. J. Rosenbrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
15. **Mayrand, D., and S. C. Holt.** 1988. Biology of asaccharolytic black-pigmented *Bacteroides* species. Microbiol. Rev. **52**:134–152.
16. **Mullins, D. E., and S. T. Rohrlich.** 1983. The role of proteinases in cellular invasiveness. Biochim. Biophys. Acta **695**:177–214.
17. **Okuda, K., J. Slots, and R. J. Genco.** 1981. *Bacteroides gingivalis*, *Bacteroides asaccharolyticus*, and *Bacteroides melaninogenicus* subspecies: cell surface morphology and adherence to erythrocytes and human buccal epithelial cells. Curr. Microbiol. **6**:7–12.
18. **Ossowski, L., J. P. Quigley, G. M. Kellerman, and E. Reich.** 1973. Fibrinolysis associated with oncogenic transformation. Requirement of plasminogen for correlated changes in cellular morphology, colony formation in agar, and cell migration. J. Exp. Med. **138**:1056–1064.
19. **Narayanan, A. S., and R. C. Page.** 1983. Connective tissues of the periodontium: a summary of current work. Coll. Rel. Res. **3**: 633–664.
20. **Pohjanpelto, P.** 1977. Proteases stimulate proliferation of human fibroblasts. J. Cell. Physiol. **91**:387–392.
21. **Pytela, R., M. D. Pierschbacher, and E. Ruoslahti.** 1985. Identification and isolation of a 140 kd cell surface glycoprotein with properties expected of a fibronectin receptor. Cell **40**:191–198.
22. **Reich, E.** 1975. Plasminogen activator: secretion by neoplastic cells and macrophages, p. 333–341. *In* E. Reich, D. P. Rifkin, and E. Shaw (ed.), Proteases and biological control. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
23. **Saksela, O.** 1981. Radial caseinolysis in agarose: a simple method for detection of plasminogen activator in the presence of inhibitory substances and serum. Anal. Biochem. **111**:276–282.
24. **Saksela, O., A. Vaheri, W. D. Schleuning, P. Mignatti, and S. Barlati.** 1984. Plasminogen activator, activation inhibitors and α_2-macroglobulin produced by cultured normal and malignant human cells. Int. J. Cancer. **33**:609–616.
25. **Salo, T., L. A. Liotta, J. Keski-Oja, T. Turpeenniemi-Hujanen, and K. Tryggvason.** 1982. Secretion of basement membrane collagen degrading enzyme and plasminogen activator by transformed cells. Role in metastasis. Int. J. Cancer **30**:669–673.
26. **Slots, J., L. Bragd, M. Wickstrom, and G. Dahlen.** 1986. The occurrence of *Actinobacillus actinomycetemcomitans*, *Bacteroides gingivalis* and *Bacteroides intermedius* in destructive periodontal disease in adults. J. Clin. Periodontal. **13**:570–577.
27. **Slots, J., and R. J. Genco.** 1984. Microbial pathogenicity. Black-pigmented *Bacteroides* species, *Capnocytophaga* species, and *Actinobacillus actinomycetemcomitans* in human periodontal disease: virulence factors in colonization, survival, and tissue destruction. J. Dent. Res. **63**:412–421.
28. **Sorsa, T., V.-J. Uitto, K. Suomalainen, H. Turto, and S. Lindy.** 1987. A trypsin-like protease from *Bacteroides gingivalis*: partial purification and characterization. J. Periodontal Res. **22**: 375–380.
29. **Stenman, S., and A. Vaheri.** 1978. Distribution of a major connective tissue protein, fibronectin, in normal human tissues. J. Exp. Med. **147**:1054–1064.
30. **Sundquist, G., G. D. Bloom, K. Enberg, and E. Johansson.** 1982. Phagocytosis of *Bacteroides melaninogenicus* and *Bacteroides gingivalis in vitro* by human neutrophils. J. Periodontal Res. **17**: 113–121.
31. **Tarone, G., G. Galetto, M. Pratt, and P. J. Comoglio.** 1982. Cell surface molecules and fibronectin mediated cell adhesion: effect of proteolytic digestion of membrane proteins. J. Cell Biol. **94**: 179–186.
32. **Teng, N., and L. Chen.** 1975. The role of surface proteins in cell proliferation as studied with thrombin and other proteases. Proc. Natl. Acad. Sci. USA **72**:413–417.
33. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from acrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **76**:4350–4354.
34. **Turto, H., S. Lindy, V.-J. Uitto, O. Wegelius, and J. Uitto.** 1977. Human leukocyte collagenase: characterization of enzyme kinetics by a new method. Anal. Biochem. **83**:557–569.
35. **Uitto, V.-J., K. Tryggvason, and T. Sorsa.** 1987. Collagenolytic enzymes in periodontal diseases. Proc. Finn. Dent. Soc. **83**:119–129.

36. **Vaheri, A., M. Kurkinen, V.-P. Lehto, E. Linder, and R. Timple.** 1978. Codistribution of pericellular matrix proteins in cultured fibroblasts and loss in transformation. Proc. Natl. Acad. Sci. USA **75**:4944–4948.
37. **Virtanen, I., T. Vartio, R. A. Badley, and V.-P. Lehto.** 1982. Fibronectin in adhesion, spreading and cytoskeletal organization of cultured fibroblasts. Nature (London) **298**:660–663.
38. **Werb, Z., and J. Aggeler.** 1987. Proteases induce secretion of collagenase and plasminogen activator by fibroblasts. Proc. Natl. Acad. Sci. USA **75**:1839–1843.
39. **Werb, Z., C. L. Mainardi, C. A. Vater, and E. D. Harris, Jr.** 1977. Endogenous activation of latent collagenase by rheumatoid synovial cells: evidence for a role of plasminogen activator. N. Engl. J. Med. **296**:1017–1023.
40. **Yamada, K. M.** 1983. Cell surface interactions with extracellular material. Annu. Rev. Biochem. **52**:761–799.